

***What is claimed:***

1. A process for preparing an acellular soft tissue graft for implantation into a mammalian system, comprising:

extracting a soft tissue sample with an extracting solution comprising one

or more nonionic detergents and one or more endonucleases, to produce

extracted tissue;

treating said extracted tissue with a treating solution comprising one or more

anionic detergents, to produce a treated tissue;

washing said treated tissue with a decontaminating solution comprising one or more

decontaminating agents to produce said acellular soft tissue graft; and

storing said acellular soft tissue graft in a storage solution comprising one or

more decontaminating agents.

2. A process for preparing commercializable quantities of acellular soft tissue grafts for implantation into mammalian systems, comprising:

obtaining tissue samples from an acceptable donor; extracting said tissue

samples with an extracting solution comprising one or more nonionic

detergents and one or more endonucleases, to produce extracted

tissue;

treating said extracted tissue with a treating solution comprising one or more

anionic detergents, to produce a treated tissue;

washing said treated tissue with a decontaminating solution comprising one

or more decontaminating agents; to produce said acellular soft tissue graft; and

storing said acellular soft tissue graft in a storage solution comprising one or more decontaminating agents.

3. A process for preparing an acellular soft tissue graft for implantation into a mammalian system, comprising:

inducing a pressure mediated flow of an extracting solution comprising

one or more nonionic detergents and one or more endonucleases, through soft tissue, to produce extracted tissue;

inducing a pressure mediated flow of a treating solution comprising one or more anionic detergents, through said extracted tissue, to produce a treated tissue;

inducing a pressure mediated flow of a decontaminating solution comprising one or more decontaminating agents through said treated tissue; to produce said acellular soft tissue graft; and

storing said acellular soft tissue graft in a storage solution comprising one or more decontaminating agents.

4. The process of claim 3, wherein said extracting solution is recirculated through said soft tissue graft.
5. The process of claim 3, wherein said treating solution is recirculated through said soft tissue graft.

6. The process of claim 3, wherein said decontaminating solution is recirculated through soft tissue graft.
7. The process of any one of claims 1, 2 or 3, said one or more decontaminating agents comprise one or more antimicrobial agents.
8. The process of any one of claims 1, 2, or 3, said extracting solution having an alkaline pH.
9. The process of any one of claims 1, 2, or 3, said extracting solution further comprises one or more organic or inorganic buffers, wherein an alkaline pH is maintained, and an osmolality of the extracting solution which is hypotonic to the cells in said soft tissue is maintained.
10. The process of any one of claims 1, 2, or 3, wherein said nonionic detergent comprise one or more detergents selected from the group consisting of: polyoxyethylene alcohol, polyoxyethylene isoalcohol, polyoxyethylene p-t-octyl phenol, polyoxyethylene nonylphenol, polyoxyethylene esters of fatty acids, and polyoxyethylene sorbitol esters.
11. The process of any one of claims 1, 2, or 3, said treating solution comprises one or more buffers selected from the group consisting of: an organic buffer and an inorganic buffer, wherein an alkaline pH is maintained, and an osmolality of the treating solution which is hypotonic to the cells in said soft tissue is maintained.
12. The process of any one of claims 1, 2, or 3, said one or more anionic detergents are selected from the group consisting of: sodium dodecylsulphate, sodium dodecylsulphonate, sodium dodecyl-N-sarcosinate, and sodium suramin.
13. The process of any one of claims 1, 2, or 3, said decontaminating solution comprises ultrapure, endotoxin-free, water solutions of antimicrobial agents, wherein said antimicrobial agents are non-reactive towards said one or more anionic detergents.

14. The process of any one of claims 1, 2, or 3, said storage solution comprises ultrapure, endotoxin-free, water.
15. The process of claim 14, wherein said storage solution further comprises one or more antimicrobial agents.
16. The process of claim 15, wherein said one or more antimicrobial agents comprise one or more members selected from the group consisting of: chlorine dioxide, ethanol, isopropanol, methanol, glycerol, and methylparaben.
17. The process of claim 16, wherein said chlorine dioxide or said methylparaben are present in said storage solution at a concentration in the range of from 0.001 % to 0.1 % (v:v).
18. The process of claim 16, wherein said ethanol, isopropanol, methanol, or glycerol are present in said storage solution at a concentration in the range of from 60% to 90% (v:v).
19. The process of claim 12, wherein said one or more anionic detergents are present in said treating solution at a concentration in the range of from 0.001% to 10% (w:v).
20. The process of claim 19, wherein said one or more anionic detergents are present in said treating solution at a concentration in the range of from about 0.08 % to about .35 % (w:v).
21. The process of any one of claims 1, 2, or 3, said one or more endonucleases comprise one or more broad spectrum endonucleases capable of degrading both deoxyribonucleic acids and ribonucleic acids.
22. The process of claim 21, wherein said one or more broad-spectrum endonucleases comprise one or more recombinant endonucleases.
23. The process of claim 22, wherein said one or more recombinant endonucleases comprise Benzonase®.

24. The process of claim 21, said one or more endonucleases are present in said extracting solution at a concentration sufficient to degrade nucleic acids present in said tissue sample.
25. The process of claim 24, wherein said one or more endonucleases are present in said extracting solution at a concentration of from about 30 IU/ml tissue to about 70 IU/ml tissue.
26. The process of claim 25, wherein said one or more endonucleases are present in said first washing solution at a concentration of about 50 IU/ml tissue.
27. The process of any one of claims 1, 2, or 3, said one or more nonionic detergents enhance activity of said one or more endonucleases.
28. The process of claim 3, said step of inducing a pressure mediated flow of extracting solution is carried out at a flow rate sufficient to carry away solutes which become dissolved in said extracting solution.
29. The process of claim 28, wherein said flow rate for said extracting solution is from about 5 mls/minute to about 220 mls/minute.
30. The process of claim 29, wherein said flow rate for said extracting solution is from about 40 mls/minute to about 90 mls/minute.
31. The process of claim 3, wherein said flow rate for said treating solution is from about 5 mls/minute to about 225 mls/minute.
32. The process of claim 31, wherein said flow rate for said treating solution is from about 40 mls/minute to about 70 mls/minute.
33. The process of any one of claims 1, 2, or 3, said treated tissue is washed with a volume of said decontaminating solution sufficient to cause an approximate 1:1000 dilution of a concentration of said treating solution.

34. The process of any one of claims 1 or 2, wherein said step of extracting is carried out for a period of time of from about 6 hours to about 24 hours.
35. The process of claim 34, wherein said step of extracting is carried out for a period of time of from about 12 hours to about 16 hours.
36. The process of any one of claims 1 or 2, wherein said step of extracting is carried out at a temperature of from about 4°C to about 42°C.
37. The process of claim 36, wherein said step of extracting is carried out at a temperature of from about 20°C to about 27°C.
38. The process of any one of claims 1 or 2, wherein said step of treating is carried out for a period of time of from about 3 hours to about 12 hours.
39. The process of claim 38, wherein said step of treating is carried out for a period of time of from about 3 hours to about 6 hours.
40. The process of any one of claims 1 or 2, wherein said step of treating is carried out at a temperature of from about 20°C to about 42°C.
41. The process of claim 40, wherein said step of treating is carried out at a temperature of from about 20°C to about 27°C.
42. The process of any one of claims 1, 2, or 3, wherein said one or more nonionic detergents are present in said extracting solution at a concentration of from about 0.1 % (w:v) to about 10% (w:v).
43. The process of claim 42, wherein said one or more nonionic detergents are present in said extracting solution at a concentration of from about 0.5% (w:v) to about 2% (w:v).

44. The process of claim 12, wherein said one or more anionic detergents are present in said treating solution at a concentration of from about 0.001% (w:v) to about 10% (w:v).
45. The process of claim 44, wherein said one or more anionic detergents are present in said treating solution at a concentration of from about 0.1% (w:v) to about .5% (w:v).
46. A decellularized soft tissue graft which is substantially non-immunogenic.
47. A decellularized soft tissue graft having tensile properties which approximate tensile properties of normal tissue, following the decellularization process.
48. The decellularized soft tissue graft of any one of claims 46 or 47, comprising a vein, an artery, or a heart valve.
49. The decellularized soft tissue graft of any one of claims 46 or 47, comprising a ligament or a tendon.
50. The decellularized soft tissue graft of any one of claims 46 or 47, comprising fascia, dura mater, pericardium or skin.
51. The decellularized soft tissue graft of claim 46, wherein said soft tissue graft remains acellular post implantation.
53. The decellularized soft tissue graft of claim 47, wherein said soft tissue graft remains acellular for at least 6 months post implantation.
54. The decellularized soft tissue graft of claim 48, wherein said soft tissue graft re-endothelializes on a luminal surface 3 to 6 months post implantation.
55. A non-immunogenic, acellular soft tissue graft produced by the process as claimed in any one of claims 1, 2, or 3.

56. A process for preparing an acellular soft tissue graft, said process comprising:

extracting one or more soft tissue samples with a hypotonic buffered extracting solution comprising one or more nonionic detergents and one or more endonucleases to produce extracted tissue;

optionally treating said extracted tissue with a treating solution

comprising a hypertonic salt solution to produce an optionally treated tissue;

treating said extracted tissue or said optionally treated tissue, with a first processing solution comprising one or more anionic detergents to produce a first processed tissue, wherein said first processing treating solution optionally comprises a hypertonic salt solution;

second optionally treating said first processed tissue with a second treating solution comprising a hypertonic salt solution to produce an optionally second treated tissue;

washing said first processed tissue or said optionally second treated tissue, in one or more washing solutions, said washing solutions comprising water and optionally one or more antimicrobial agents, to produce washed tissue; and

storing said washed tissue in a storage solution comprising one or more decontaminating agents and water.

57. The process of claim 56, said extracting solution comprises one or more organic or inorganic buffers, wherein said buffers maintain said extracting solution at an alkaline pH and maintain a hypotonic osmolality of said extracting solution which is hypotonic to cells in said one or more soft tissue samples.



58. The process of claim 57, said extracting solution comprises one or more nonionic detergents selected from the group consisting of: polyoxyethylene alcohol (Brij series, Lubrol W, AL series), polyoxyethylene isoalcohol (Sterox AJ, AP series, Emulphogen BC series, Renex 30 series), polyoxyethylene p-t-octyl phenol ( Triton X series, Igepal CA series Nonidet P 40), polyoxyethylene nonylphenol (Triton N series, Igepal CO series, Surfonic N series), polyoxyethylene esters of fatty acids (Sterox CO series, Myrj series, Span series), and polyoxyethylene sorbitol esters (Tween series, Emasol series).
59. The process of claim 58, said extracting solution further comprises one or more endonuclease.
60. The process of claim 56, said first processing solution comprises one or more organic or inorganic buffers, wherein said buffers maintain said extracting solution at an alkaline pH and maintain a hypertonic osmolality of said extracting solution which is hypertonic to cells in said one or more soft tissue samples.
61. The process of claim 60, said first processing solution comprises one or more anionic detergents selected from the group consisting of sodium dodecylsulphate, sodium dodecylsulphonate, sodium dodecyl-N-sarcosinate, sodium laurylsulfate, and sodium suramin.
62. The process of claim 56, said hypertonic salt solution comprises water solutions comprising one or more members selected from the group consisting of a monovalent salt, a divalent salt, and an antimicrobial agent, wherein said hypertonic salt solution is reactive with said one or more anionic detergents.

63. The process of claim 56, said storage solution comprises ultrapure, endotoxin-free, water.
64. The process of claim 56, said one or more decontaminating agents comprise one or more antimicrobial agents.
65. The process of claim 64, said one or more antimicrobial agents selected from the group consisting of: chlorine dioxide, ethanol, isopropanol, methanol, glycerol, and methylparaben.
66. The process of claim 65, wherein a concentration of chlorine dioxide or methylparaben is in a range of from 0.001 % to 0.1 % (v:v).
67. The process of claim 65, wherein a concentration of ethanol, isopropanol, methanol, or glycerol is in a range of from 60% to 90% (v:v).
68. The process of claim 61, wherein a concentration of said one or more anionic detergents are in a range of from 0.001% to 10% (w:v).
69. The process of claim 68, said concentration of anionic detergent is 0.24% (w:v), and said second processing solution further comprising 0.5M sodium chloride.
70. The process of any one of claims 56 or 59, said endonuclease is a broad spectrum endonuclease which degrades deoxyribonucleic acids (DNA) and ribonucleic acids (RNA).
71. The process of claim 70, said broad-spectrum endonuclease is a recombinant endonuclease.
72. The process of claim 70, said recombinant endonuclease is Benzonase.
73. The process of claim 70, wherein a concentration of said broad-spectrum endonuclease is sufficient to degrade deoxyribonucleic acids and said ribonucleic acids present in said soft tissue sample having a volume in an allotted time.

74. The process of claim 73, said concentration of said broad-spectrum endonuclease is from about 20 IU to about 400 IU per 1 ml volume of tissue.
75. The process of claim 70, wherein an activity of said broad-spectrum endonuclease is enhanced by said one or more nonionic detergents.
76. The process of claim 56, said extracting comprises subjecting said soft tissue sample to a pressure mediated flow of said extracting solution.
77. The process of claim 56, said optionally treating comprises subjecting said extracted tissue to a pressure mediated flow of said treating solution.
78. The process of claim 56, said treating comprises subjecting said extracted tissue or said optionally treated tissue to a pressure mediated flow of said first processing solution.
79. The process of claim 56, said second optionally treating comprises subjecting said first processed tissue to a pressure mediated flow of said second treating solution.
80. The process of claim 56, said washing comprises subjecting said second optionally treated tissue or said first processed tissue to a pressure mediated flow of said one or more washing solutions.
81. The process of claim 76, wherein a flow rate of said extracting solution is sufficient to remove any solutes dissolved in said extracting solution.
82. The process of claim 81, said flow rate of said extracting solution is from about 5 mls/minute to about 100 mls/minute.
83. The process of claim 82, said flow rate of said extracting solution is from about 30 mls/minute to about 60 mls/minute.

84. The process of claim 77, wherein a flow rate of said first processing solution is from about 5 mls/minute to about 100 mls/minute.
85. The process of claim 84, said flow rates of said first processing solution is from about 30 mls/minute to about 60 mls/minute.
86. The process of claim 62, said hypertonic salt solution comprises one or more members selected from the group consisting of: sodium chloride, potassium chloride, lithium chloride, calcium chloride, sodium phosphate, calcium hydroxide, potassium sulfate, lithium sulfate, calcium phosphate, potassium phosphate, lithium phosphate, ammonium chloride, magnesium chloride, and calcium sulfate.
87. The process of claim 56, said extracting is carried out for a time period of from about 6 hours to about 24 hours.
88. The process of claim 87, said extracting is carried out for a time period of from about 12 hours to about 16 hours.
89. The process of claim 56, said extracting is carried out at a temperature of from about 4° C to about 42° C.
90. The process of claim 89, said extracting is carried out at a temperature of from about 20° C to about 27° C.
91. The process of claim 56, said optionally treating is carried out for a time period of from about 3 hours to about 24 hours.
92. The process of claim 91, said optionally treating is carried out for a time period of from about 3 hours to about 6 hours.

93. The process of claim 31, said optionally treating is carried out for a time period of at least about 3 hours.
94. The process of claim 56, said optionally treating is carried out at a temperature of from about 20°C to about 42° C.
95. The process of claim 94, said optionally treating is carried out at a temperature of from about 20° C to about 27° C.
96. The process of claim 58, said nonionic detergents are at a concentration of from about 0.001% (w:v) to about 10% (w:v).
97. The process of claim 96, said nonionic detergents are at a concentration of from about 0.1% (w:v) to about 2% (w:v).
98. The process of claim 61, said anionic detergents are at a concentration of from about 0.001% (w:v) to about 4% (w:v).
99. The process of claim 98, said anionic detergents are at a concentration of from about 0.1% (w:v) to about 2% (w:v).
100. The process of claim 86, said hypertonic salt solution is at a concentration of from about 0.00001 M to about 2.0 M.
101. The process of claim 100, said hypertonic salt solution is at a concentration of from about 0.0002 M to about 0.08 M.
102. The process of claim 86, said monovalent salt and said divalent salt comprise one or more calcium salts selected from the group consisting of: calcium chloride, calcium hydroxide, magnesium chloride, lithium chloride, potassium chloride, and sodium chloride.

103. An acellular tissue graft, comprising a soft tissue sample substantially free from cellular elements produced by the process as claimed in claim 102, wherein recellularization of said acellular tissue graft *in vivo* or *in vitro*, is retarded.
104. An acellular tissue graft, comprising: a soft tissue sample substantially free from cellular elements, and calcium ion precipitated anionic detergent.
105. An acellular tissue graft, comprising a soft tissue sample substantially free from cellular elements and calcium ion precipitated anionic detergent, produced by the process as claimed in claim 102, wherein recellularization of said acellular tissue graft *in vivo* or *in vitro*, is retarded.
106. The acellular tissue graft of any one of claims 104 or 105, said calcium ion precipitated anionic detergent is present in an amount of at least 1.0  $\mu$ mole/mg wet weight of tissue.
107. The acellular tissue graft of claim 106, said calcium ion precipitated anionic detergent is present in an amount of from about 0.1 wt% to about 10.0 wt%.
108. An acellular tissue graft of claim 56, said anionic detergent is SDS and is present at a concentration of about 0.001 wt%, wherein said acellular tissue graft contains comparable levels of SDS as when concentrations of about 1.0 wt% of a different anionic detergent is used.
109. A process for preparing an acellular soft tissue graft, said process comprising:  
extracting one or more soft tissue samples with a hypotonic buffered extracting solution at an alkaline pH, comprising one or more nonionic detergents and one or more endonucleases to produce extracted tissue;  
treating said extracted tissue with a treating solution comprising a hypertonic salt

solution to produce a treated tissue;  
treating said treated tissue, with a first processing solution comprising one or more anionic detergents in a hypertonic salt solution to produce a first processed tissue;  
second treating said first processed tissue with a second treating solution comprising a hypertonic salt solution to produce a second treated tissue;  
washing said second treated tissue, in one or more washing solutions, said washing solutions comprising water and optionally comprising one or more antimicrobial agents, to produce washed tissue; and  
storing said washed tissue in a storage solution comprising one or more decontaminating agents and water.

110. An acellular tissue graft, comprising: a soft tissue sample substantially free from cellular elements produced by the process as claimed in any of claims 56 or 109, wherein said acellular tissue graft retains dimensions approximate to said soft tissue sample prior to processing.
111. The acellular tissue graft of claim 110, wherein said extracting solution comprises 0.24wt% sodium dodecylsulfate in 0.5 M sodium chloride.
112. An acellular tissue graft, comprising: a soft tissue sample substantially free from cellular elements produced by the process as claimed in any of claims 56 or 108, wherein said acellular tissue graft retains tensile properties approximate to tensile properties of said soft tissue sample prior to processing.

113. The acellular tissue graft of claim 112, said soft tissue sample is a heart valve, and wherein said acellular heart valve leaflets maintain normal coaptation.
114. The acellular tissue graft of claim 110, wherein tissue retaining lower amounts of SDS and/or CaDS, recellularizes more quickly *in vivo* and/or *in vitro* than tissues retaining higher amounts of SDS and/or CaDS.
115. An acellular tissue graft, comprising a soft tissue sample substantially free from cellular elements, and precipitated anionic detergent present in an amount effective to enhance recellularization of said acellular tissue *in vivo* or *in vitro*.
116. An acellular tissue graft, comprising a soft tissue sample substantially free from cellular elements, and precipitated anionic detergent present in an amount effective to enhance recellularization of said acellular tissue *in vivo* or *in vitro*, produced by the process as claimed in claim 102, wherein recellularization of said acellular tissue *in vivo* or *in vitro* is enhanced.
117. The acellular tissue graft of any one of claims 115 or 116, said precipitated anionic detergent is present at a concentration of no more than 20 wt%.
118. The acellular tissue graft of claim 117, said precipitated anionic detergent is present at a concentration of from about 0.2 wt% to about 2.0 wt%.
119. A method for modulating recellularization of an acellular soft tissue graft upon implantation of said acellular soft tissue graft in a mammalian system, comprising: precipitating an amount of anionic detergent in a tissue sample, said amount precipitated is effective to enhance or retard recellularization of said acellular soft tissue graft.



120. An acellular soft tissue graft, comprising: a soft tissue sample essentially free from cellular elements and precipitated anionic detergent, produced by the process as claimed in claim 119.